Effects of Acoustic Power

Ultrasound pressure waves of current B-mode imaging machines typically destroy microbubbles. Fixed concentrations of the contrast agent are stabilized in cellulose dialysis tubes to determine the characteristics of unmodified and surface-modified GOAM in a commercial diagnostic ultrasonic field. The two different ultrasound transducers described above are used to acquire the RF data and B-mode images. The acoustic power of the ultrasound machine as reflected by the mechanical index ("MI") provided on the machine is set in 0.1 MI steps, from 0.2 to 0.8 MI. The tube contains fresh contrast agents for each MI level test for each transducer. The backscatter coefficient for each step for each contrast agent is determined.

Effects of Suspension Condition

Different suspension conditions affect the properties of ultrasound contrast agents. The effects are tested by changing different air concentrations, diluting the contrast agent, and utilizing different carrier media.

The effects of air concentration are assessed for both unmodified and surface-modified GOAM at fixed concentrations using the methods described in Sboros. (Sboros et al., "An In Vitro Comparison of Ultrasonic Contrast Agents in Solutions with Varying Air Levels," *Ultrasound in Med. & Biol.*, 26:807-18 (2000) which is incorporated by reference herein). Sterile water is used as the suspension medium. A sterile bag filled with sterile water is infused with helium or air to achieve partial oxygen pressures (pO_2) of 1.5 or 24.7 kPa, respectively. These suspensions are injected slowly in the cellulose dialysis tubing. The imaging data is gathered under these conditions using the Aloka 5500 PHD RF machine to

Banner & Witcoff, Ltd.
Ten South Wacker Drive
Chicago, IL 60606-7407

acquire the RF data and B-mode images. Microbubble concentration and size are determined for the suspensions. Normalized ultrasonic backscatter vs. concentration is examined.

In vitro characterization of ultrasonic contrast media conducted in aqueous solutions do not necessarily adequately simulate the behavior of contrast agent in the circulatory system. Therefore, different concentration levels of GOAM are suspended with sterilized water, saline, plasma and whole blood at 37°C. The contrast agent is suspended in an imaging cell similar to that described by Lazewatsky and colleagues. (Lazewatsky et al., "The Effect of Dilution on the Measurement of In-vitro Properties of Ultrasound Contrast Agents," *Proceeding of 1999 IEEE Ultrasonics Symposium*, 1737-42 (1999) which is incorporated by reference herein). The two different ultrasound transducers described above are used to acquire the RF data and B-mode images. Time-video intensity along with backscatter data are acquired using the video and RF data acquisitions systems described above.

Attenuation as a function of frequency

The *in vitro* enhancement and attenuation properties of unmodified and surface-modified GOAM are examined using the methods described by de Jong (de Jong and Hoff, "Ultrasound Scattering Properties of Albunex® Microspheres," *Ultrasonics*, 31(3) 175-81 (1993) which is incorporated by reference herein) and by Forsberg et al. (Forsberg et al., "In Vio Evaluation of a New Contrast Agent," *Proceeding of 1994 IEEE Ultrasonics Symposium*, 1555-58 (1994); "Quantitative Acoustic Characterization of a New Surfactant-Based Ultrasound Contrast Agent," *Ultrasound in Med. & Biol.*, 23:1201-08 (1997) which both are incorporated by reference herein) using a flow pump to provide flow through the dialysis

Banner & Witcoff, Ltd.
Ten South Wacker Drive
Chicago, IL 60606-7407

tubing in the imaging tank described above. Frequency dependent dose attenuation is determined, as well as the time-attenuation dose dependence curves.

Sound velocity

Because unmodified and surface-modified GOAM have particulate gadolinium encased within the microbubble, it is important to determine the effects of the embedded gadolinium on microbubble sound velocity. Different concentrations of GOAM are utilized for this test. A modified version of the displacement method described by Hall et al. (Hall et al., "Experimental Determination of Phase Velocity of Perfluorocarbons: Application to Targeted Contrast Agents," *IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control*, 47:75-84 (2000) which is incorporated by reference herein) is employed. The imaging tank described above is employed along with the cellulose tubing. A polished stainless steel plate is placed 0.5 cm behind the cellulose tube. The two different ultrasound transducers described above are used to acquire the RF data and B-mode images. The tube is filled with sterilized water. Then the tube is filled with different concentrations of the contrast agents. Full rinsing is conducted between each injection of different contrast media. Sound velocity for each concentration is determined.

Scattering

Because unmodified and surface-modified GOAM have particulate gadolinium encased within the microbubble, it is also important to determine the effects of the embedded gadolinium on microbubble directional scattering. Different concentrations of GOAM are utilized for this test. The two different ultrasound transducers described above are used to acquire the RF data and B-mode images The cellulose tube is filled with sterilized water first.

Banner & Witcoff, Ltd.
Ten South Wacker Drive
Chicago, IL 60606-7407